Investigation of the Chemical Composition of Saliva for the Purpose of Diagnostics of Oral Cavity Diseases

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(Received August 21, 2007; revised March 3, 2008)

Abstract

Elemental composition of 30 samples of oral liquid from four groups of patients living in the Omsk Region was investigated by means of atomic emission spectral analysis with inductively coupled plasma. Comparative analysis of the composition of saliva for patients with different diseases of oral cavity showed that the elemental composition is specific in each case. It was established that the diagnostics of the corresponding diseases may be carried out on the basis of the data on the ratios Ca/P and Na/K. It was discovered with the help of mathematical statistics that the distribution of microelements Zn, Cu, Fe, Mn, Al also has group-related features.

Key words: hydroxyapatite, microelements, dental calculi, spectral analysis, diagnostics, discriminant analysis

INTRODUCTION

Chemical and physicochemical analysis methods are widely used during the recent years to study the role of elements present in biological objects. In particular, investigations of the elemental composition of pathogenic biomineral formations are of interest for determination of possible participation of microelements in their genesis [1–4]. Additional data on the mechanisms and factors of calculus formation, as well as other pathological processes taking place in a human organism may be obtained through the studies of the composition of biological liquids, for example saliva.

Saliva plays an important part in sustaining the physiological equilibrium of mineralization and demineralization processes in dental enamel. Saliva is present in oral cavity as a thin film of liquid on various surfaces. It is known [5–8] that the diseases of oral cavity arise as a consequence of disturbed equilibrium in the system dental enamel–saliva under the action of various unfavourable factors.

The role of microelements, including metals, in the origin of different pathological states of the oral cavity, in particular the formation of dental calculi, has not been studied completely till present. However, it is known that many microelements take part in metabolism. Investigation of biogenic elements (K, Ca, Mn, Fe, Co, Cu, Zn *etc.*) is of major interest because investigation of biological liquids will allow one to establish the effect of ecological conditions of habitation on some groups of diseases.

The goal of the present work was to investigate the features of elemental composition of oral liquid with the help of atomic emission spectroscopy with inductively coupled plasma, and to reveal group differences in the cases of pathological processes in human oral cavity.

EXPERIMENTAL

We studied 28 samples of oral liquid from patients at an age within the range 18–30, given by the city dental health polyclinic No. 1 of Omsk. The samples of oral liquid were taken from patients in the morning on an empty stomach and before tooth brushing. The elemental composition of oral liquid was determined by means of AES-ICP (Optima 2000 DV, Perkin Elmer). This method was chosen because it is widely used to study the elemental composition of biological objects [9]. The reliability of results was checked using chemical and physicochemical methods of analysis: Ca^{2+}, Mg^{2+} were determined by means of chelatometric titration, Na^+, K^+ by means of potentiometry, PO_4^{3-}, Zn^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Al^{3+} by means of photometry [10, 11].

The results were processed using the software of the spectrometer. Quantitative calculation involved the calibration plot method. The sensitivity of the method is 10^{-8}–10^{-2} mass %, error is 3–7 rel. %.

RESULTS AND DISCUSSION

Previously we carried out the panoramic X-ray fluorescence analysis using the synchrotron radiation (SR-XFA) to determine the microelement composition of dental calculi from patients living in the Omsk Region [4]. It was determined that the studied samples of dental calculi contain 14 microelements – Ti, V, Mn, Fe, Ni,

TABLE 1

Characterization of the elemental composition of the samples of oral liquid

Sample*	Content, mg/L								
	Ca	P	Na	K	Zn	Cu	Fe	Al	Mn
I – 1	20	140	160	690	0.38	0.033	1.4	–	–
I – 2	112	200	370	710	3.2	1.7	0.44	–	–
I – 3	18	120	310	840	0.73	0.081	0.40	–	–
I – 4	13	130	170	640	1.3	0.014	0.43	–	–
I – 5	42	280	340	1400	2.2	0.07	0.63	–	–
I – 6	39	410	320	730	1.5	–	1.2	–	0.010
I – 7	106	260	290	860	0.89	0.10	0.21	–	–
I – 8	79	170	180	960	1.1	0.063	0.35	0.16	0.006
II – 1	16	290	120	1200	0.24	0.017	–	–	–
II – 2	45	200	170	850	0.84	0.19	0.16	–	–
II – 3	24	140	150	710	0.18	0.10	0.11	–	–
II – 4	27	240	140	1200	1.2	0.055	0.33	–	–
II – 5	13	130	130	720	0.16	0.038	0.13	–	–
III – 1	37	100	120	410	0.70	0.07	0.50	–	0.073
III – 2	38	57	200	410	0.11	0.01	0.02	0.046	–
III – 3	54	97	230	690	0.32	0.008	–	–	0.023
III – 4	38	120	170	610	0.30	0.093	0.15	0.029	–
III – 5	47	110	300	790	1.5	0.013	–	0.024	–
III – 6	58	140	240	730	0.32	0.021	0.10	–	0.045
III – 7	78	190	68	810	0.20	0.028	0.10	0.064	0.016
III – 8	53	130	330	670	0.18	0.02	0.28	0.028	0.017
III – 9	95	170	210	760	0.37	0.017	0.12	0.047	0.041
IV – 1	51	110	130	430	0.36	0.54	0.20	0.80	0.047
IV – 2	69	160	130	570	3.7	0.39	1.2	0.77	0.065
IV – 3	55	120	190	570	0.44	0.29	0.21	0.61	0.053
IV – 4	59	200	44	670	0.51	0.93	0.28	0.64	0.032
IV – 5	45	130	120	560	0.78	0.11	0.28	–	–
IV – 6	55	190	320	1000	1.6	0.093	0.28	–	–

*The first figure is the number of a group; the second is the number of a patient.

Cu, Zn, Br, Rb, Zr, Ag, Sn, I, Ba. The high content of a number of elements in the studied samples is likely to be due to their isomorphous implantation into apatite, which is the basic mineral component of human dental calculi.

On the basis of the results of analyses, the content of the following elements in the oral cavity was determined: Ca, P, Na, K, Mg (the elements that form the mineral basis of calculi) and Zn, Cu, Fe, Mn, Al (microelements exhibiting the maximal content in the studied samples of dental calculi).

Four groups were distinguished during the investigation: group I – patients having dental deposit in the oral cavity (8 persons, 28.6 %), group II – patients with dental caries (5 persons, 17.9 %), group III – patients with necrosis of the hard tooth tissues, (9 persons, 32.1 %), and group IV – reference (6 persons, 21.4 %). The reference group included the persons with the same somatic status "practically healthy", stable against diseases, and having no diseases of parodentium and mucous coat of oral cavity.

The elemental composition of the samples of oral liquid was determined by means of AES-ICP (Table 1).

In order to process the array of the data obtained, we used discriminant analysis method (Statistics 6.0, StatSoft) that allows one to show graphically the difference between the groups under investigation (Fig. 1). One can see that the data on the content of the nine elements under determination turned out to be close to each other for the groups with dental caries and with dental calculi in oral cavity; these data even overlap each other partially, similarly tot eh data for the group with necrosis of the hard tooth tissues and the reference group. The most clearly exhibited differences are observed for the reference group and the group of patients with dental deposit in oral cavity.

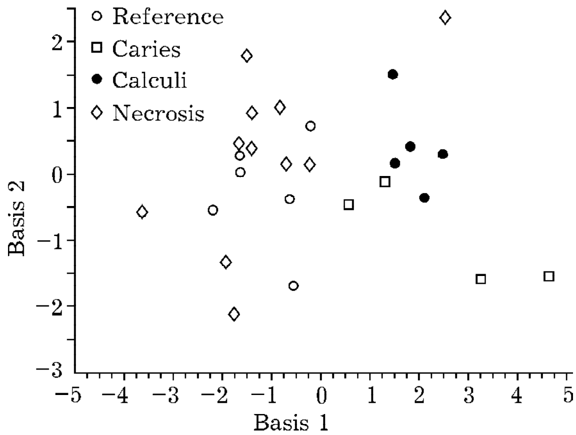

Fig. 1. Diagram of separation of the groups of diseases under investigation.

Additional investigation of the array of the initial data by means of multidimensional correlation analysis allowed us to establish a number of regularities concerning the concentrations of separate elements. For example, correlations in the concentrations of potassium and phosphorus were detected ($\alpha = 0.05$, $r = 0.61$), as well as iron, copper and zinc) $\alpha = 0.05$, $r = 0.69$), which allows us to reveal the regularities of the distribution of these elements over the groups under investigation.

Confidence intervals were calculated for the basic elemental composition for each group under investigation (Table 2).

One can see in these data (see Tables 1, 2) that the appearance of various diseases in oral cavity causes substantial deviations of ion content in oral liquid from the normal values. For example, the formation of dental calculi is ac-

TABLE 2

Average content of elements in the samples of oral liquid for the groups under investigation, mg/L

Element	Groups			
	I	II	III	IV
	($n = 8$, $t = 2.37$)	($n = 5$, $t = 2.78$)	($n = 9$, $t = 2.31$)	($n = 6$, $t = 2.57$)
Calcium	24±9.2	25±13	55±15	56±8.5
Phosphorus	$(2.2\pm0.83)\cdot10^2$	$(2.0\pm0.66)\cdot10^2$	$(1.3\pm0.30)\cdot10^2$	$(1.5\pm0.38)\cdot10^2$
Sodium	$(2.7\pm0.71)\cdot10^2$	$(1.4\pm0.22)\cdot10^2$	$(2.1\pm0.63)\cdot10^2$	$(1.6\pm0.32)\cdot10^2$
Potassium	$(8.5\pm1.9)\cdot10^2$	$(9.4\pm2.4)\cdot10^2$	$(6.5\pm1.2)\cdot10^2$	$(6.3\pm1.9)\cdot10^2$

Note. Here and in Table 3: n is the number of samples, t is Student coefficient ($\alpha = 0.05$).

companied by a decrease in calcium ion content, but an increase in the content of phosphorus and electrolytic components – sodium and potassium ions – occurs. In the case of caries, the same changes are observed, but sodium content remains at he normal level. In the case of necrosis of the hard tooth tissues, no essential deviations from the normal values were revealed, but differences in microelemental content are observed for Zn, Cu, Fe, Mn, Al.

The mineralizing function of saliva is to a high extent due to the electrolytic components because the major substance of enamel is hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. Due to this fact, enamel is capable of reversible isomorphous substitution of its ions for the ions from the oral liquid without sharp distortions of the crystal structure and without sharp changes of properties.

It was established [12] that the mineralizing function of the oral liquid is realized due to its supersaturation with Ca^{2+} and HPO_4^{2-} ions. The basic mechanism through which supersaturation of saliva with these ions is sustained is provided by their micellar state. The nucleus of a micelle is composed of $Ca_3(PO_4)_2$, potential determining ions are HPO_4^{2-}, counter ions are Ca^{2+}, the latter are also incorporated into the diffuse layer. The micelles of $Ca_3(PO_4)_2$ are the structural units of oral liquid; its mineralizing function depends on their stability. In the oral cavity, the micelles are protected from aggregation mainly by glycoprotein mucin with its high surface activity which makes it able to get sorbed on colloid particles thus exhibiting protective action.

Dominant cations of saliva (Na and K) along with other ions provide the osmotic pressure of saliva, its ionic force, and are incorporated into the salt components of buffer systems. The physiological concentrations of sodium and potassium ions in the oral liquid are high, so they keep an important part in regulating homeostatic mechanisms in the system dental enamel–saliva.

Taking into account the substantial role of Ca, P, Na and K in physiological processes that take part in oral cavity, let us consider the distribution of these elements in the groups under investigation.

Thus, in the samples taken from patients with dental deposit, the concentration of sodium ions is maximal ($\alpha < 0.05$), while in the samples taken from the group of patients with dental caries and dental calculi the concentration of potassium ions exceeds the normal level essentially. An increase in the concentration of electrolytic components including Na and K up to the values exceeding the limits of physiological norm promotes the transition of ions from the diffuse layer to the adsorption layer and the disappearance of the diffuse layer. These micelles are in the isoelectric state because the charge of their granules becomes equal to zero, they lose stability and coagulate.

It is known [13] that a substantial increase in the concentration of the dominant cations of saliva leads to weakening of the protective properties of biopolymers due to the destruction of the hydrate shells of the macromolecules of high-molecular compounds (HMC) and their denaturation. Destruction of the hydrate shells of HMC occurs due to hydration of sodium and potassium cations. So, an increase in the concentration of the dominating cations of saliva (Na and K), on the one hand, causes a decrease in the stability of $Ca_3(PO_4)_2$ micelles, on the other hand, weakens the protective properties of HMC. This causes the loss of the protective mechanisms sustaining supersaturation with Ca^{2+} and HPO_4^{2-} ions, and therefore leads to the distortion of structural and mineralizing properties of saliva.

The ions Na^+ and K^+ conditioning the native conformations of HMC are connected with their macromolecules; they get liberated in the case when the structural properties of saliva are distorted, because a decrease in the biological activity of HMC is accompanied by weakening of eh bonds with electrolytes [13]. This is evidenced by an increase in the concentration of active Na^+ and K^+ ions under unfavourable conditions in the oral cavity.

The concentration of calcium ions in the samples of oral liquid taken from the group of patients with dental calculi and dental caries is reliably lower than the normal level ($\alpha < 0.05$), while phosphorus content is slightly higher. Saliva gets less saturated with Ca and P, cases to be mineralizing liquid and turns into demineralising one.

In the case if pH of saliva is 6.4 and lower, which corresponds to the isoelectric points of its proteins, distortion of the native conformations of proteins occurs, the biological activity decreases, and the ability to protect salivary micelles from aggregation is lost. So, acidification of saliva leads to distortion of the protective mechanisms that sustain the mineralising components in supersaturated state, which can cause the rise and development of caries process.

For pH of saliva equal to 7.0 and higher, distortion of the process of micelle formation is caused by the fact that Ca^{2+} and PO_4^{3-} ions cannot be present simultaneously in the adsorption layer because they form a difficultly soluble compound $Ca_3(PO_4)_2$. The participation of the microorganisms of dental deposit, manifested as the capture of precipitated $Ca_3(PO_4)_2$ and its transfer to the rough surface of dental enamel, creates favourable conditions for the formation of dental calculi [12].

The concentrations of calcium, phosphorus, sodium and potassium in the studi9ed samples of oral liquid changed in opposite directions, that is why we used the ratios Ca/P and Na/K in order to increase the significance of differences. The results after statistical processing are shown as confidence intervals (Table 3).

So, using the Ca/P ratio we may distinguish the groups of patients with caries and calculi formation in oral cavity on the basis of the substantial deviation of the parameters from the normal level. The Na/K ratio may be used to separate the groups of patients with caries and with calculi formation: for Na/K < 0.2 the risk of dental caries is high, while for Na/K > 0.2 the risk of dental calculi formation is high.

The application of the cluster analysis method (Statistica 6.0, StatSoft) allows us to study the distribution of microelements Zn, Cu, Fe, Mn, Al over the studied groups of patients (Fig. 2). Separation into three groups is observed: the first group includes mainly the samples taken from the patients with the normal state of oral cavity (Nos. 1–5), the second one includes the patients with calculi formation in orla cavity (Nos. 11–15), the third – with necrosis of the hard tooth tissues (Nos. 16–23). The samples taken from patients with dental caries (Nos. 6–10) are uniformly distributed over the three groups. Such a separation of the initial data array into clusters allows us to demonstrate the difference between the considered groups of patients and to assume that microelements play an essential part in genesis or development of definite diseases. For example, under the conditions of calculi formation in oral cavity, zinc and iron content increases, while necrosis of the hard tooth tissues is accompanied by the lower content of all the microelements under investigation than the normal level.

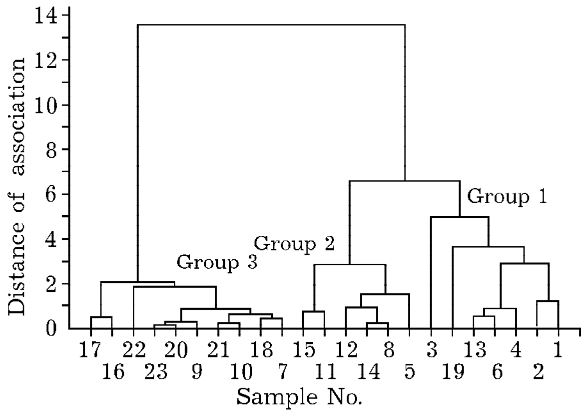

Fig. 2. Tree of association of the samples of oral liquid with respect to the microelement composition.

In general, rather high concentrations of Zn, Cu, Fe, Mn, Al in the oral liquid of patients from the Omsk Region may be explained by the local conditions and the state of environment [4]. According to the data of the Ob-Irtysh interregional department of hydrome-

TABLE 3

Data on the ratios Ca/P, Na/K for the samples of oral liquid under investigation

Ratio	Groups			
	I	II	III	IV
	(n = 8, t = 2.37)	(n = 5, t = 2.78)	(n = 9, t = 2.31)	(n = 6, t = 2.57)
Ca/P	0.11±0.02	0.13±0.07	0.46±0.09	0.39±0.08
Na/K	0.33±0.10	0.16±0.03	0.33±0.09	0.24±0.09

teorology and environmental monitoring, Irtysh River is most heavity polluted with the compounds of manganese (44 MPC), iron (24 MPC), copper (23 MPC), zinc (14 MPC), while the concentrations of manganese and copper exceed 100 MPC in the Om River.

CONCLUSION

The collection of oral liquid samples taken from patients living in the Omsk Region was studied by means of AES-ICP. The features of the distribution of elements over four groups of patients were revealed; a procedure for the diagnostics of some diseases of human oral cavity on the basis of the elemental composition of saliva was proposed.

In addition, the same method may be used to reveal predisposition to definite diseases of oral cavity and to carry out the diagnostics at an early stage, which is important for determining the effect of environmental factors on human health, as well as prophylactics and treatment of the corresponding diseases.

REFERENCES

1 O. A. Golovanova, P. A. Pyatanova, N. N. Strunina, B. T. Baysova, *Zh. Prikl. Spektrosc.,* 70, 3 (2003) 432.
2 T. G. Krymova, V. N. Balin, V. V. Kolkutin, Yu. A. Kostitsyn, *Stomatologiya,* 1 (2007) 4.
3 A. A. Korago, Vvedeniye v Biomineralogiyu, St. Petersburg, 1998, pp. 144–172.
4 O. A. Golovanova, L. V. Belskaya, N. Yu. Berezina, *Prikl. Spektrosc.,* 73, 6 (2006) 792.
5 O. Yu. Puzikova, Prognozirovaniye Razvitiya Kariesa Zubov s Uchetom Integrirovannykh Pokazateley i Matematicheskogo Modelirovaniya (Candidate's Dissertation in Medicine), Omsk, 1999, p. 150.
6 E. V. Borovskiy, V. K. Leontiev, Biologiya Polosti Rta, Meditsina, Moscow, 1991, p. 271.
7 T. L. Pilat, *Stomatologiya,* 3 (1984) 88.
8 M. V. Galiulina, Elektrolitnye Komponenty Smeshannoy Slyuny Cheloveka v Usloviyakh Fiziologii i Patologii Polosti Rta (Author's Abstract of Candidate's Dissertation in Biology), Moscow, 1988, p. 26.
9 M. Thompson and D. N. Walsh, Handbook on Spectrometry Analysis with Inductively Coupled Plasmas [Russian translation], Nedra, Moscow, 1988, p. 174.
10 O. A. Golovanova, E. A. Nemchinova, M. A. Borisenko *et al.*, Mineralogiya Tekhnogeneza, Miass, 2005, pp. 27–29.
11 A. N. Pitaeva, A. P. Korshunov, V. G. Suntsov *et al.*, Fiziko-khimicheskiye Metody Issledovaniya Smeshannoy Slyuny v Klinicheskoy i Eksperimentalnoy Stomatologii, Omsk, 2001, p. 71.
12 V. K. Leontiev, M. V. Galiulina, *Stomatologiya,* 5 (1991) 17.
13 R. Chang, Physical Chemistry with Applications to Biological Systems, Collier Macmillan Ltd., NY, 1977.